DISSERTATION ON

A STUDY ON SYSTEMIC INFLAMMATION AND RISK OF CARDIOVASCULAR DISEASES IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Submitted in partial fulfilment of
Requirements for

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MADRAS MEDICAL COLLEGE

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CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY ON SYSTEMIC INFLAMMATION AND RISK OF CARDIO VASCULAR DISEASES IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE"** submitted by **Dr.SUNIL KUMAR. S** appearing for Part II M.D. Branch I General Medicine Degree examination in March 2008 is a bonafide record of work done by him under my direct guidance and supervision in partial fulfillment of regulations of the Tamil Nadu Dr. M.G.R. Medical University, Chennai. I forward this to the Tamil Nadu Dr.M.G.R. Medical University, Chennai, Tamil Nadu, India.

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DECLARATION

I solemnly declare that the dissertation titled "**A STUDY ON SYSTEMIC INFLAMMATION AND RISK OF CARDIO VASCULAR DISEASES IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE"** is done by me at Madras Medical College & Govt. General Hospital, Chennai during 2006-2007 under the guidance and supervision of **Prof.D.RAJASEKARAN, M.D.**

The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University towards the partial fulfillment of requirements for the award of M.D. Degree (Branch I) in General Medicine.

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CONTENTS

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a major cause of chronic morbidity and mortality throughout the world. It is defined as preventable and treatable disease with some significant extra pulmonary effects that may contribute to the severity in individual patients[1]. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases[1].

The impact of COPD on an individual patient depends on the severity of symptoms (especially breathlessness and decreased exercise capacity), systemic effects, and any co morbidities the patient may have—not just on the degree of airflow limitation.

There is growing evidence for systemic inflammation in COPD. Increased circulating levels of inflammatory cytokines and acute phase proteins occur in stable disease, and COPD exacerbations are notably associated with pulmonary and systemic inflammation[2].

COPD is an important risk factor for atherosclerosis[3-5]. Even modest reductions in expiratory flow volumes elevate the risk of ischemic heart diseases, strokes, and sudden cardiac deaths 2- to 3-fold, independent of other risk factors[3-7]. Indeed, poor lung function has been shown to be a better predictor of all-cause and cardiac-specific mortality than established risk factors such as serum cholesterol[5]. Cardiovascular conditions are the leading cause of mortality among those with impaired lung function[3, 5, 7]. The mechanism or mechanisms responsible for this association, however, remain largely unknown

Although the pathogenesis of atherothrombosis is complex and multifactorial, persistent low-grade systemic inflammation is believed to be one of the centerpieces in effecting clot formation[8].

The association of COPD with systemic inflammation and cardiac injury are analyzed in our study population.

OBJECTIVES OF THE STUDY

1. To determine whether COPD is associated with increased circulating levels of CRP and other inflammatory markers.

2. To determine whether the intensity of the systemic inflammation is associated with the severity of airflow obstruction.

3. To find out whether systemic inflammation and COPD are associated with cardiac injury.

REVIEW OF LITERATURE

Chronic Obstructive Pulmonary Disease (COPD) is a major cause of chronic morbidity and mortality throughout the world. Many people suffer from this disease for years and die prematurely from it or its complications. COPD is the fourth leading cause of death in the world[9], and further increases in its prevalence and mortality can be predicted in the coming decades[10].

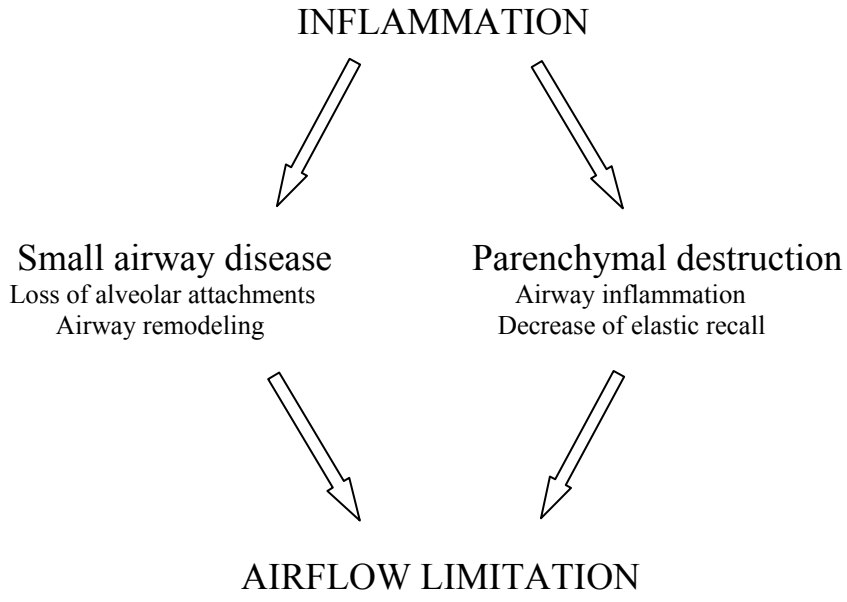
Definition: According to Global initiative for chronic obstructive lung disease (GOLD[1]), COPD is a preventable and treatable disease with some significant extra pulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases[1]. The term "emphysema" and "chronic bronchitis," are not included in this definition. Emphysema, or destruction of the gas exchanging surfaces of the lung (alveoli), is a pathological term that is often (but incorrectly) used clinically and describes only one of several structural abnormalities present in patients with COPD[1]. Chronic bronchitis, or the presence of cough and sputum production for at least 3 months in each

of two consecutive years, remains a clinically and epidemiologically useful term[1].

Worldwide, cigarette smoking is the most commonly encountered risk factor for COPD, although in many countries, air pollution resulting from the burning of wood and other biomass fuels has also been identified as a COPD risk factor[1].

The chronic airflow limitation characteristic of COPD is caused by a mixture of small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema), the relative contributions of which vary from person to person[1].

Mechanisms Underlying Airflow Limitation in COPD



SPIROMETRIC CLASSIFICATION OF SEVERITY

Spirometry is essential for diagnosis and provides a useful description of the severity of pathological changes in COPD. Spirometry should be performed after the administration of an adequate dose of an inhaled bronchodilator (e.g., 400 mcg salbutamol) in order to minimize variability[11].

Spirometric Classification of COPD
Severity Based on Post-Bronchodilator FEV1[1]

Stage I: Mild	FEV1/FVC < 0.70 FEV1 ≥80% predicted
Stage II: Moderate	FEV1/FVC < 0.70 50% ≤FEV1 < 80% predicted
Stage III: severe	FEV1/FVC < 0.70 30% ≤FEV1 < 50% predicted
Stage IV: Very Severe	FEV1/FVC < 0.70 FEV1 < 30% predicted or FEV1 < 50% predicted plus chronic respiratory failure.

SYMPTOMS OF COPD

The characteristic symptoms of COPD are chronic and progressive dyspnea, cough, and sputum production. Chronic cough and sputum production may precede the development of airflow limitation by many years. Conversely, significant airflow limitation may develop without chronic cough and sputum production.

STAGES OF COPD

Stage I: Mild COPD - Characterized by mild airflow limitation (FEV1/FVC < 0.70; FEV1 ≥80% predicted).Symptoms of chronic cough and sputum production maybe present, but not always[1].

Stage II: Moderate COPD - Characterized by worsening airflow limitation (FEV1/FVC < 0.70; 50% ≤FEV1 < 80%predicted), with shortness of breath typically developing on exertion and cough and sputum production sometimes also present[1].

Stage III: Severe COPD - Characterized by further worsening of airflow limitation (FEV1/FVC < 0.70; 30% ≤FEV1< 50% predicted), greater shortness of breath, reduced exercise capacity, fatigue, and repeated exacerbations that almost always have an impact on patients' quality of life[1].

Stage IV: Very Severe COPD - Characterized by severe airflow limitation (FEV1/FVC < 0.70; FEV1 < 30% predicted *or* FEV1 < 50% predicted plus the presence of chronic respiratory failure). Respiratory failure is defined as arterial partial pressure of oxygen (PaO2) less than 8.0 kPa (60 mm Hg) with or without arterial partial pressure of CO2 (PaCO2) greater than 6.7 kPa (50 mm Hg) while breathing air at sea level. Respiratory failure may also lead to effects on the heart such as cor pulmonale (right heart failure). At this stage, quality of life is very appreciably impaired and exacerbations may be life threatening[1].

EPIDEMIOLOGY

COPD is under diagnosed and under treated, resulting in underestimation of the burden of this disease[12]. The prevalence of COPD is highest in countries where cigarette smoking, for example, is still very common[13]. Prevalence data based on the presence of airflow limitation provide an accurate estimate of the burden of clinically significant COPD[14].

Two large epidemiologic studies, in which the diagnosis of COPD was established using spirometry, evaluated COPD prevalence in 2005. In a nationwide Korean survey involving 9,243 subjects, Kim and colleagues reported that the prevalence of COPD, determined by criteria of the GOLD,

was 17.2% among subjects older than 45 years[15]. Prevalence increased with increasing age, especially in males, in those with more than 20 pack-years of smoking, and in low-income subjects. Most of the COPD found was mild to moderate (FEV1 > 50%), Menezes and colleagues reported wide variability in COPD prevalence between five major cities in Latin America[16].

RISK FACTORS FOR COPD

1) Genes: polygenic and hereditary deficiency of alpha-I antitrypsin[17].

2) Exposure to particles

   - Tobacco smoke[18, 19.]
   - Occupational dusts, organic and inorganic[20-23].
   - Indoor air pollution from heating and cooking with biomass in poorly vented dwellings[24-30].
   - Outdoor air pollution.

3) Decreased Lung Growth and Development[31].

4) Oxidative stress.

5) Gender: prevalence is more in males compared to females. However in developed countries prevalence is almost equal[32-33].

6) Age: prevalence increases with increase in age.

7) Respiratory infections.

8) Socioeconomic status: risk of COPD is inversely related to socioeconomic status[34].

9) Nutrition.

10) Co morbidities.

PATHOLOGICAL CHANGES

Proximal airways - *Inflammatory cells:* Macrophages and CD8+ T cells.

Structural changes: Goblet cells, enlarged submucosal glands and squamous metaplasia of epithelium[35].

Peripheral airways - *Inflammatory cells:* Macrophages, T lymphocytes (CD8+ > CD4+), B lymphocytes and fibroblasts.

Structural changes: Airway wall thickening, peribronchial fibrosis, luminal inflammatory exudates and airway narrowing[36].

Lung parenchyma - *Inflammatory cells:* Macrophages, CD8+ T lymphocytes.

Structural changes: Alveolar wall destruction, apoptosis of epithelial and endothelial cells, Centrilobular and Panacinar emphysema[37].

Pulmonary vasculature - *Inflammatory cells:* Macrophages, T lymphocytes

Structural changes: pulmonary hypertension[38].

PATHOGENESIS

Amplification of the normal inflammatory response of the respiratory tract to chronic irritants appears to be seen in COPD.

Inflammatory Cells: COPD is characterized by recruitment of neutrophils, macrophages, and lymphocytes[39] .These cells release inflammatory mediators and interact with structural cells in the airways and lung parenchyma.

Inflammatory Mediators: Chemotactic factors like leukotriene B4 and interleukin-8, Proinflammatory cytokines like TNF-alpha, IL-1beta, and IL-6, and Growth factor TGF-ß which are released from the inflammatory cells are responsible for inflammation[40].

Oxidative Stress: Oxidative stress may be an important amplifying mechanism in COPD[41]. Oxidants are generated by cigarette smoke and other inhaled particulates and released from activated inflammatory cells. Oxidative stress has several adverse consequences in the lungs, including activation of inflammatory genes, inactivation of antiproteases, stimulation of mucus secretion, and stimulation of increased plasma exudation.

Protease-Antiprotease Imbalance: There is compelling evidence for an imbalance in the lungs of COPD patients between proteases and antiproteases[1].There is increase in proteases such as Neutrophil elastase, Cathepsin G, Proteinase 3, Cathepsins B, K, L, S and matrix metalloproteinases like MMP-8, MMP-9 and MMP-12.This is associated with decrease in antiprotinases like alpha-1 antitrypsin, alpha-1 antichymotrypsin, Elafin, Cystatins and Tissue inhibitors of MMP 1-4.

PATHOPHYSIOLOGY

1) Airflow Limitation and Air Trapping: The peripheral airway obstruction due to inflammation, fibrosis, and luminal exudates leads to progressive air trapping during expiration resulting in hyperinflation. Hyperinflation increases functional residual capacity which results in dyspnea and limitation of exercise capacity.

2) **Gas Exchange Abnormalities:** results in hypoxemia and Hypercapnia.

3) Mucus Hypersecretion: is due to mucous metaplasia with increased numbers of goblet cells in response to chronic airway irritation resulting in chronic productive cough.

4) Pulmonary Hypertension: pulmonary hypertension may develop late in the course of COPD and is due to hypoxic vasoconstriction and structural changes in small pulmonary arteries[42].

SYSTEMIC FEATURES

It is increasingly recognized that COPD involves several systemic features, particularly in patients with severe disease[43, 44].

Systemic Features of COPD

- Systemic inflammation
- Cachexia: loss of fat free mass
- Skeletal muscle wasting: apoptosis, disuse atrophy
- Osteoporosis
- Depression
- ormochromic normocytic anemia
- Increased risk of cardiovascular disease: associated with CRP[45].

SYSTEMIC INFLAMMATION IN COPD

Circulating Inflammatory Cells

Many studies of COPD have reported changes in various inflammatory cells, including neutrophils and lymphocytes, in peripheral blood. The activation of peripheral blood neutrophils, resulting in potentiation of cytotoxic and migratory responses, has also been shown. Noguera and colleagues investigated the production of reactive oxygen species and the expression of surface adhesion molecules in circulating neutrophils of patients with stable COPD[46, 47]. Compared with control subjects, patients with stable disease showed an increased expression of CD11b/CD18 in circulating neutrophils with lower expression levels of ICAM-1. Increased plasma soluble ICAM-1, a surrogate of its expression on the endothelium, was reported by others. In addition, Noguera and coworkers showed that blood neutrophils isolated from patients with COPD produced more reactive oxygen species under basal conditions as well as after stimulation in vitro, as compared to neutrophils from smoking and nonsmoking control subjects and this respiratory burst correlated with the elevated expression of adhesion molecules[47]. In another study, Burnett and colleagues demonstrated that peripheral neutrophils isolated from patients

with COPD showed enhanced chemotaxis and extracellular proteolysis in vitro[48, 49]. In contrast, Cataldo and coworkers found no differences in the secretion of matrix metalloproteinase (MMP)-9 by circulating granulocytes comparing patients with COPD and control subjects[50]. The expression of stimulatory Ga, a G protein subunit that is a key signaling protein for cell adhesion and activation in circulating neutrophils, has been shown to be downregulated irrespective of the clinical condition of the patient. However, the pathogenic implications of most of these findings are still unclear, and need confirmation in well characterized patient groups.

Recent findings also indicate abnormal lymphocyte function in COPD. Increased activity of cytochrome oxidase was reported in the lymphocytes of patients with COPD compared with healthy subjects [51], and found to be significantly related to disease severity as reflected by the degree of airflow limitation. In a recent study, Hageman and colleagues [52] investigated activation of nuclear enzyme poly ADP-ribose polymerase-1 (PARP-1), which forms extensive poly ADP-ribose polymers from its substrate NAD + after activation by reactive oxygen species–induced DNA strand breaks. Activation of PARP-1 in peripheral blood lymphocytes of patients with COPD was more prevalent than in lymphocytes of healthy, age-matched control subjects, supporting a contribution of PARP-1

activation to the pathophysiology of COPD. PARP-1 activation was associated with a reduction of the NAD+ status, the consequences of which can include impaired production of high-energy phosphates.

In contrast to numerous studies showing a decreased pulmonary CD4+/CD8+ ratio in COPD, this has not to date been studied as extensively in the systemic compartment. Several reports suggest that cigarette smoke alone may trigger a shift in the numbers of CD4+ and CD8+ lymphocytes, which may be reversible after smoking cessation. In this respect De Jong and coworkers reported no significant differences between lymphocyte subsets in peripheral blood of patients with COPD and healthy smokers[53]. However, these authors also found that, within the group of nonsmokers, the percentage of CD8+ cells was significantly higher in subjects with COPD compared with control subjects and the CD4:CD8 ratio correlated positively with higher FEV1 values. Additional studies are necessary to understand better the contribution of circulating lymphocytes to the pathogenesis of COPD.

The propensity of circulating monocytes to release proinflammatory molecules as a possible factor in a systemic inflammatory response was evaluated recently in stable COPD. Monocytes isolated from patients with

COPD release significantly more MMP-9 but less IL-8 than control subjects[54]. Cell stimulation resulted in a larger enhancement of IL-6 and MCP-1 release from COPD monocytes, whereas monocytes from healthy individuals released higher levels of ICAM-1. As a consequence of cellular inflammatory changes, different authors have reported changes in the oxidant/antioxidant balance in the systemic circulation. Markedly reduced Trolox-equivalent antioxidant capacity (TEAC) of plasma, as well as increased levels of lipid peroxidation products, both indices of overall oxidative stress, are seen in healthy smokers and patients with COPD versus nonsmoking control subjects[55, 56]. Hageman and coworkers demonstrated a significant reduction of TEAC of deproteinized plasma as well as a reduction of plasma uric acid in patients with stable COPD when compared with control subjects[52]. Further evidence of persistent systemic oxidative stress in patients with COPD was provided by the finding of higher levels of isoprostane F2alpha -II, a stable prostaglandin isomer formed by reactive oxygen species-dependent peroxidation of arachidonic acid, in the urine of patients with COPD versus smoking control subjects[57]. Together these studies indicate that both smoking and COPD are associated with significant systemic oxidative stress.

Inflammatory Mediators in Plasma: During the last decade, several studies investigating systemic manifestations of COPD have reported enhanced levels of circulating inflammatory mediators, such as acute-phase reactants and cytokines. The acute-phase proteins are liver-derived, key players in innate immunity and reduction of inflammatory reactions. Schols and colleagues demonstrated increased levels of C-reactive protein and lipopolysaccharide binding protein in patients with stable COPD[58], and which was most pronounced in a subset of patients with COPD with an increased resting energy expenditure and decreased fat-free mass. The lack of a response to some intervention strategies such as nutritional therapy seems to be related to the level of this systemic inflammatory response. A prospective epidemiological study in a cohort of 8,955 subjects from a Danish general adult population study revealed that increased plasma levels of fibrinogen, another acute-phase reactant, are associated with reduced lung function and increased risk of COPD, independent of smoking status[59]. The rise in the systemic levels of acute-phase proteins suggests that hepatocytes are activated to produce these reactants, although increasing evidence indicates that other tissue specific cells such as lung epithelial cells are also able to produce acute-phase proteins. The formation of acute-phase reactants is induced strongly by cytokines such as IL-6 or TNF-alpha .Indeed,

enhanced circulating levels IL-6 and TNF-alpha have been reported in COPD[52]. These increased levels of proinflammatory mediators are not counterbalanced by up regulation of anti-inflammatory mediators like soluble IL-1RII in patients with stable COPD[60]. Yasuda and colleagues demonstrated that plasma levels of soluble Fas (CD95), an inhibitor of apoptosis, were increased significantly in severe COPD when compared to healthy control subjects and patients with mild/moderate COPD[61]. Upregulation of apoptotic pathways, TGF-β, TNF-α and Fas in peripheral blood in patients with COPD was associated with increased T cell death. Future studies are needed to assess whether these systemic changes are present continuously as part of the stable state in COPD or reflect day-to-day variations in the inflammatory state.

ORIGIN OF SYSTEMIC INFLAMMATION

The origin of the systemic inflammation present in patients with COPD remains poorly understood, and several (independent) pathways may be involved. As smoking causes many important extrapulmonary effects, like cardiovascular diseases, tobacco smoke alone may contribute significantly to systemic inflammation in COPD. In this respect, both systemic oxidative stress and peripheral vascular endothelial dysfunction

were reported in passive smokers and in smokers with only few pack-years. A second possible mechanism is the involvement of the local pulmonary inflammatory response in systemic inflammation, which was investigated recently by Vernooy and coworkers[62]. Comparison of levels of sTNF-R or IL-8 in sputum and plasma did not reveal direct correlations, thereby suggesting that the systemic inflammatory response in mild-to-moderate COPD is not due to an overflow of Inflammatory mediators from the pulmonary compartment but, rather, that the inflammatory processes in the local and systemic compartment are regulated differently. Further evidence for the hypothesis that the inflammatory processes in the airways and the systemic circulation are independent processes comes from recent studies of Michel and colleagues[63].

A third pathway involved in the process of systemic inflammation could be related to hypoxia, a recurrent problem in COPD. As studies in vitro have revealed that hypoxia results in enhanced cytokine production by macrophages, the systemic hypoxemia observed in patients with COPD may contribute to the activation of the TNF system. Indeed, significant inverse correlations between PaO2 and circulating TNF-alpha and sTNF-R levels in patients with COPD were reported[64]. Alveolar macrophages appear to have a prominent role in the inflammatory response in hypoxia-induced lung injury

and the related up regulation of inflammatory mediators. Based on the observation of decreased antioxidative capacity of skeletal muscles in COPD, Rabinovich and coworkers have demonstrated an abnormal cytokine response to moderate exercise in patients with COPD. Recently, it was reported that local muscle exercise, despite the increased oxidative stress, did not result in changes in plasma cytokine levels. Further research is needed to clarify the origin and physiological significance of these exercise-related inflammatory responses in COPD.

CARDIOVASCULAR DISEASES AND COPD

Chronic obstructive pulmonary disease (COPD) is an important risk factor for atherosclerosis[3-5]. Even modest reductions in expiratory flow volumes elevate the risk of ischemic heart diseases, strokes, and sudden cardiac deaths 2-to 3-fold, independent of other risk factors[3-7]. Indeed, poor lung function has been shown to be a better predictor of all-cause and cardiac-specific mortality than established risk factors such as serum cholesterol[5]. Cardiovascular conditions are the leading cause of mortality among those with impaired lung function[3, 5, 7]. The mechanism or mechanisms responsible for this association, however, remain largely unknown. Of course tobacco smoking is a shared risk factor for both COPD and cardiovascular disease. Yet it is possible that others factors may increase the Cardiovascular risk of patients with COPD even further.

Although the pathogenesis of atherothrombosis is complex and multifactorial, persistent low-grade systemic inflammation is believed to be one of the centerpieces in effecting clot formation[8]. Compelling epidemiological data link systemic inflammation to atherosclerosis, ischemic heart disease, strokes, and coronary deaths[65,66].These observations have been strongly supported by biomedical experiments that show the direct effects of

certain inflammatory markers, such as C-reactive protein (CRP), on the pathogenesis of plaque formation[67,68].

Sin DD et al[69] showed that systemic inflammation is present in moderate and severe COPD and airflow obstruction is an important risk factor for cardiac injury. They also showed that in the presence of elevated CRP, the risk increases almost 2-fold, which suggests an important interplay of systemic inflammation with airflow obstruction in the development of ischemic heart disease. Sin et al showed that in COPD patients, every 10% reduction in FEV1 equate to an increase in cardiovascular mortality of 28%.Huiart et al[70] using Saskatchewan Health databases showed that Cardiovascular morbidity and mortality rates were higher in the COPD cohort than in the general population. More hospitalizations for CVD than for COPD itself were reported. Cardiovascular disease and more specifically ischemic heart disease were reported as a more frequent cause of death than COPD itself. Miller et al and sin et al[69] showed that CRP and cardiovascular infarction injury score increases as the severity of COPD increases. COPD patients have increased arterial stiffness compared to healthy controls as measured by applanation tonometry. Endothelial dysfunction and arterial stiffness may also contribute to adverse cardiovascular events in COPD.

The epidemiologic evidence linking COPD and cardiovascular morbidity and mortality is strong. Even after adjustments for traditional cardiovascular risk factors such as serum total cholesterol, hypertension, obesity and smoking, patients with COPD have a two- to threefold increase in the risk of cardiovascular events including death[71].

But there remain many unanswered questions. First, there is a scarcity of studies that evaluated COPD as a potential effect modifier of cardiovascular events in individuals with pre-existing heart disease. Second, it is not clear whether COPD amplifies the adverse effects of cigarette smoking on the cardiovascular system or whether it acts predominantly via the smoke–heart disease causal pathway. Third, the mechanisms through which COPD contributes to atherosclerosis and, ultimately, cardiovascular events are not fully known. Finally, it is not clear whether anti-COPD interventions, aside from smoking cessation, can modify cardiovascular risks in COPD.

MATERIALS AND METHODS

SETTINGS

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ETHICAL APPROVAL

Obtained

STUDY DESIGN

To evaluate the presence of systemic inflammation in COPD patients and whether both are associated with cardiac injury, a cross sectional study design was chosen.

PERIOD OF STUDY

May 2006 TO June 2007

SAMPLE SIZE

Cases: 70, Controls: 30

INCLUSION CRITERIA

1. Patients with symptoms suggestive of COPD.
2. Patients with age more than 45 years.
3. Patients not on corticosteroids.

EXCLUSION CRITERIA

1. Patients with cor pulmonale
2. Patients with pulmonary tuberculosis
3. Patients with diabetes mellitus
4. Patients known to have systemic hypertension
5. Patients who are unable to perform spirometry
6. Critically ill patients
7. Patients having elevated lipid profile
8. Patients having angina, cardiac or renal failure

STUDY POPULATION

Of the 100 patients enrolled for the study who attended out patient clinic of Institute of Internal Medicine, Government General hospital,50

patients were selected as cases and 30 as controls after thorough history taking and clinical examination. Other 20 patients were excluded as per exclusion criteria.

The patients having following criteria [according to GOLD[1]] were defined as having COPD

1. The presence of cough and sputum production for at least 3 months in each of two consecutive years.

2. Exertional dyspnoea.

3. Physical examination showing (a) signs of airflow limitation like prolonged expiration and expiratory wheeze which is not fully reversible. (b) Signs of hyperinflation.

4. Spirometry showing post-bronchodilator FEV1/FVC ratio < 0.70.

The present analysis was restricted to participants >45 years of age who performed spirometry that met acceptability and reliability criteria of the American Thoracic Society[72]. This age cutoff was chosen in order to improve the diagnostic accuracy of using airflow obstruction as a marker of COPD.

For each enrolled subject, the personal and family medical histories were obtained. On the study day, height and weight were measured twice during the examination. Weight was measured to the nearest 100 g with bare foot. Height was measured to the nearest mm with a stadiometer. Body mass index (BMI) was calculated by the formula

$$BMI = \text{Weight (kg)} / \text{Height (m)}^2$$

Blood pressure was measured was measured with patient in the sitting position after a 5 min rest, with mercury sphygmomanometer (cuff size 12.5 × 40 cm). The systolic BP and diastolic BP were read to the nearest 2 mmHg. Disappearance of Korotkoff's sounds (phase 5) was the criterion for diastolic BP. Total amount of smoking was determined by calculating Pack-years of smoking. Presence of co morbidities was determined by participants' responses to the question: "Has the doctor ever told you had diabetes (hypertension, tuberculosis, congestive heart failure, etc)?"

SPIROMETRY

Spirometry was performed with equipment that met the American Thoracic Society performance criteria[72]. To adjust for height, age, sex, and race, published prediction equations for forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC) was used[73]. FEV1–FVC ratio of<0.70 was used to define airflow obstruction. Mild, moderate, and severe airflow obstruction were defined as FEV1 of <80% of predicted (equivalent to Stage 1 of the [GOLD] classification), 50% to 80% of predicted (Stage 2a), and <50% of predicted (Stages 2b and 3), respectively[1].

ELECTROCARDIOGRAPHY

Standard 12 lead ECG was obtained from each patients. An ECG coding scheme was then applied to the data to calculate a Cardiac Infarction Injury Score (CIIS) for each participant[74]. These scores were used to estimate the participants' risk of underlying ischemic heart disease. A CIIS score of ≥20 denoted probable infarction, ≥15 possible infarction and ≥10 borderline abnormality[74].

LABAROTARY MEASUREMENTS

The hs-CRP level was measured by using latex-enhanced nephelometry[75].

Hs-crp values ranges between 0.3 and 8.6 mg/l in healthy men and between 0.2 and 9.1mg/l in healthy women who are not taking hormone replacement therapy. The recommendation of Centre for Disease Control and prevention (CDC) and the American Heart Association (AHA) for interpretation of hs-CRP results was taken into consideration[76]. It states that hs-CRP of

<1 mg/l	low risk
1to 3 mg/l	Average risk
>3 mg/l	High risk.

Overnight fasting (at least 10 hours) blood specimen were obtained for measurement of serum lipids. Concentration of total cholesterol, HDL-cholesterol, and triglycerides were assessed enzymatically with commercially available reagents. Concentration of LDL- cholesterol was calculated by use of the Friedewald equation for participants who had triglycerides (< 400 mg/dl)

LDL = TC ─ HDL-c ─ TGL/5.

Total leukocyte count, differential count, platelets, serum creatinine, blood urea, serum electrolytes, and other biochemical evaluation were performed according to routine standards, at biochemical lab attached to Institute of Biochemistry, Government General Hospital.

FINANCIAL SUPPORT: nil.

CONFLICT OF INTEREST: nil.

STATISTICAL ANALYSIS

Statistical analysis was carried out for 80 participants [50 COPD patients, 30 controls] after categorizing each variable. Base line data was collected from patients without and with mild, moderate and severe obstruction. Age, sex, smoking in pack years, blood pressure, body mass index, spirometric results, total leukocyte count, differential count, platelets, CRP and CIIS were analyzed.

The significance of difference in means between two groups were analyzed using One way ANOVA F-test and the significance of difference in proportions by Chi-square test. Multiple comparisons were done by Bonferroni t-test. The correlation between CIIS and severity of COPD was done by spearman's rho methods.

Statistical significance was taken when p value < 0.05. Statistical analysis was carried out using standard formulae. Microsoft excel 2003 and SPSS (statistical package for social sciences) version 13 software was used for data entry and analysis.

RESULTS

There were totally 80(68.8% males, 31.3% females) participants in the study, out of which 50 (62.5%) had airflow obstruction on their spirometry. 16 (32%) had mild, 13(26%) had moderate and 21(42%) had severe obstruction.

Table 1: Age Distribution in years

	N	Mean	Std. Deviation	Oneway ANOVA F-test
Normal	30	55.47	5.794	F=0.78 P=0.51 Not significant
Mild	16	56.69	6.008	
Moderate	13	59.08	8.361	
Severe	21	56.43	8.675	
Total	80	56.55	7.096	

The average age of participants was 56.55 years. There was no statistical significance (p=0.51) between increase in age and severity of airflow Obstruction.

Table 2: Sex distribution

	Sex				Chi-square test
	Male		Female		
	n	%	n	%	
Normal	15	50.0%	15	50.0%	
Mild	9	56.3%	7	43.8%	$\chi 2=16.02$
Moderate	10	76.9%	3	23.1%	P=0.001
Severe	21	100.0%	0	0.00%	significant
Total	55	68.8%	25	31.3%	

Out of 50 participants who had airflow obstruction 80% were males and 20% were females. Male sex was significantly associated with airflow obstruction (mild, moderate and severe) compared to female sex (p=0.001).

Table 3: Smoking status

	Smoker				Chi-square test
	No		Yes		
	n	%	n	%	
Normal	24	80.0%	6	20.0%	
Mild	10	62.5%	6	37.5%	$\chi 2=29.68$
Moderate	5	38.5%	8	61.5%	P=0.001
Severe	1	4.8%	20	95.2%	significant
Total	40	50.0%	40	50.0%	

All smokers were males in the study. Smoking status was a positive risk factor for moderate and severe airflow obstruction (p=0.001).

Table 4: Smoking in pack years

	N	Mean	Std. Deviation	Oneway ANOVA F-test
Normal	6	21.267	2.0027	F=2.91 P=0.05 significant
Mild	6	17.083	5.5715	
Moderate	8	37.250	35.8589	
Severe	20	38.777	19.0856	
Total	40	27.591	21.3482	

Severity of airflow obstruction was significantly associated with number of years of smoking. Severity increased as the number of pack years of smoking increased and it was statistically significant (p=0.05).

Table 5: **Body Mass Index (kg/m^2)**

	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison By Bonferroni t-test
Normal	30	24.930	2.0024	F=16.56 P=0.001 significant	1 Vs 2,3,4
Mild	16	20.299	2.7299		
Moderate	13	21.046	3.7362		
Severe	21	19.749	3.5349		
Total	80	22.013	3.6760		

The average BMI of the participants was 22.013 kg/m^2.It was inversely correlated with lung function. Compared to the controls, BMI of the cases was low and as the severity of airflow increased, BMI reduced, which was statistically significant (p=0.002).

Table 6: Systolic blood pressure in mm of Hg

	N	Mean	Std. Deviation	Oneway ANOVA F-test
Normal	30	125.87	6.410	F=1.56 P=0.21 Not significant
Mild	16	128.00	5.750	
Moderate	13	130.15	9.326	
Severe	21	123.90	12.609	
Total	80	126.48	8.912	

There was no significant differences in systolic blood pressure between the cases and controls (p=0.21).

Table 7: Diastolic blood pressure in mm of Hg

	N	Mean	Std. Deviation	Oneway ANOVA F-test
Normal	30	76.33	7.576	
Mild	16	77.88	5.439	F=1.34
Moderate	13	80.77	7.049	P=0.27
Severe	21	78.29	6.141	Not significant
Total	80	77.88	6.790	

Diastolic blood pressure between cases and controls was also not statistically significant (p=0.27).

Table 8: Leukocytes per mm^3

	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison By Bonferroni t-test
Normal	30	6046.67	783.332	F=36.47 P=0.001 significant	1 Vs3,4 2Vs 3,4 3 Vs 1,2 4Vs 1,2
Mild	16	6593.75	711.307		
Moderate	13	7623.08	485.032		
Severe	21	8090.48	814.804		
Total	80	6948.75	1131.818		

Compared to control, cases with moderate and severe airflow obstruction were having elevated levels of leukocytes (p=0.001), where as significant difference was not found between control and cases with mild obstruction. The highest levels were observed in the group with severe airflow obstruction.

Table 9: Neutrophils per mm^3

	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison By Bonferroni t-test
Normal	30	3952.97	539.330	F=31.88 P=0.001 significant	1 Vs3,4 2Vs 3,4 3 Vs 1,2 4Vs 1,2
Mild	16	4295.38	445.713		
Moderate	13	4924.92	443.059		
Severe	21	5414.81	688.065		
Total	80	4563.13	815.125		

The circulating levels of neutrophils were higher in participants with than in those without airflow obstruction (p=0.001). The highest levels were observed in the group with severe airflow obstruction. The increase in leukocyte count in this group was driven largely by the neutrophilic subpopulation of cells.

Table 10: Lymphocytes per mm^3

	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison By Bonferroni t-test
Normal	30	1842.10	319.097	F=17.87 P=0.001 significant	1 Vs3,4 2Vs 3,4 3 Vs 1,2 4Vs 1,2
Mild	16	1904.69	470.044		
Moderate	13	2388.23	239.409		
Severe	21	2465.86	335.273		
Total	80	2107.10	445.732		

The circulating levels of lymphocytes was also higher in cases with moderate and severe airflow obstruction compared to controls and cases with mild obstruction (p=0.001).There was no statistically significant differences between the latter two groups.

Table 11: Platelets per mm^3

	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison By Bonferroni t-test
Normal	30	2.3143	.19833	F=33.89 P=0.001 significant	1 Vs3,4 2Vs 3,4 3 Vs 1,2,4 4Vs 1,2,3
Mild	16	2.2813	.28159		
Moderate	13	2.5662	.11608		
Severe	21	2.8643	.22087		
Total	80	2.4930	.32035		

Similarly the circulating levels of platelets was also higher in cases with moderate and severe airflow obstruction compared to controls and cases with mild obstruction (p=0.001).There was no statistical differences between the latter two groups. Highest levels were noted in severe obstruction group.

Table 12: Hs-CRP in mg/l

	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison By Bonferroni t-test
Normal	30	3.453	1.7965	F=64.66 P=0.001 significant	1 Vs,3,4 2Vs 1,4 3 Vs 1,4 4Vs 1,2,3
Mild	16	7.400	1.3049		
Moderate	13	8.631	.9050		
Severe	21	12.067	3.4706		
Total	80	7.345	4.0783		

The levels of hs-CRP was significantly elevated in cases with moderate and severe airflow obstruction compared to control and cases with mild obstruction (p=0.001).Among cases significant differences were noted between mild and severe and also between moderate and severe obstruction groups. Not much difference was noted between mild and moderate obstruction groups.

Table 13 : CARDIOVASCULAR INFARCTION INJURY SCORE (CIIS)

	N	Mean	Std. Deviation	Oneway ANOVA F-test	Multiple comparison By Bonferroni t-test
Normal	30	5.30	1.622	F=50.21 P=0.03 significant	1 Vs 3,4 2 Vs 3,4 3 Vs 1,2 4 Vs 1,2
Mild	16	7.00	2.658		
Moderate	13	10.15	2.075		
Severe	21	13.57	3.385		
Total	80	8.60	4.181		

There were important differences in ECG findings among the four lung function groups. Compared with the control group, moderate, and severe airflow obstruction were associated with an increase in CIIS of 4.85+_0.453, and 8.27+_1.76 U, respectively which was statistically significant (p=0.03). Even though group with mild obstruction had elevated CIIS compared to control, it was not statistically significant.

We assessed the strength of correlation of the CIIS with BMI and inflammatory markers. We found that CIIS was positively correlated with leukocytes, platelets and CRP, and negatively correlated with BMI.

Table 14: Correlations

	SBP mm hg	**DBP mm hg**	**BMI Kg/m^2**	**LEUKO CYTES per mm^3**	***LYMPHO CYTES* per mm^3**	**NEUTR OPHILS per mm^3**	**PLATELETS per mm^3**	**CRP mg/l**
CIIS	-.179	.105	-.481(**)	.669(**)	.448(**)	.664(**)	.548(**)	.675(**)
	.112	.353	.000	.000	.000	.000	.000	.000
	80	80	80	80	80	80	80	80

0-0.2 poor correlation. 0.2-0.4 fair.

0.4-0.6 moderate. 0.6-0.8 substantial.

0.8-1.0 good.

Fig 1: Age distribution

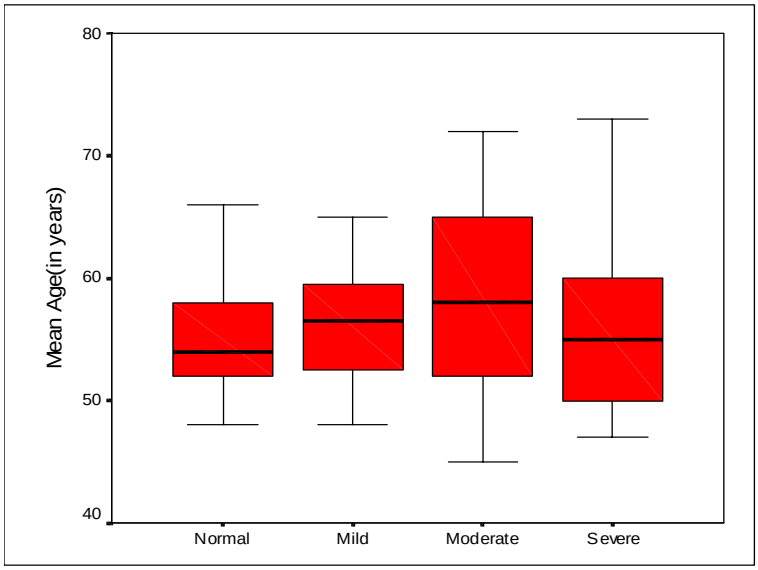


Fig 2: Sex distribution

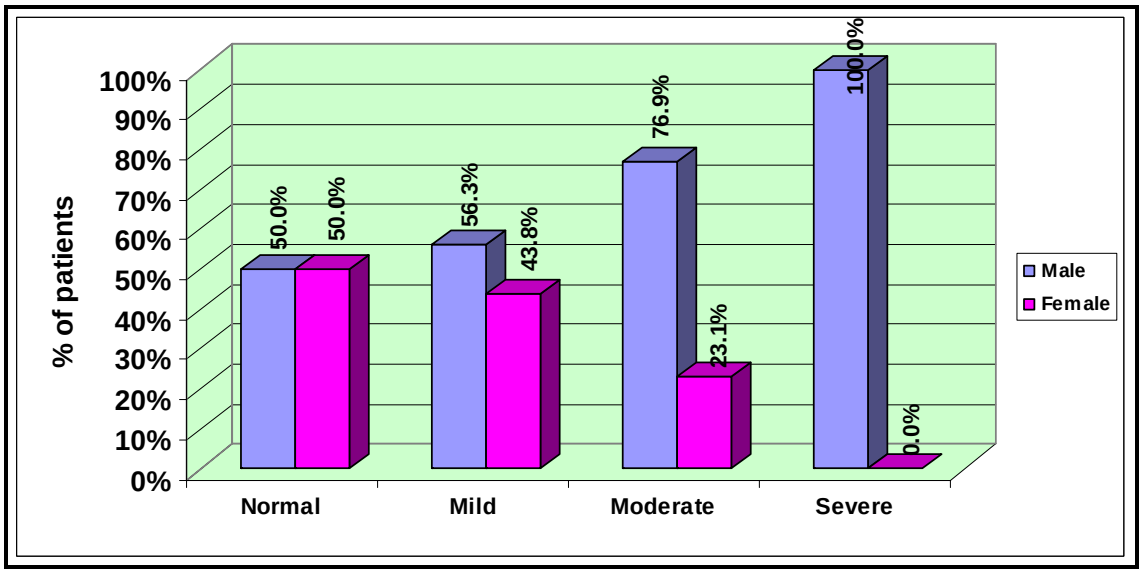


Fig 3: Smoking status

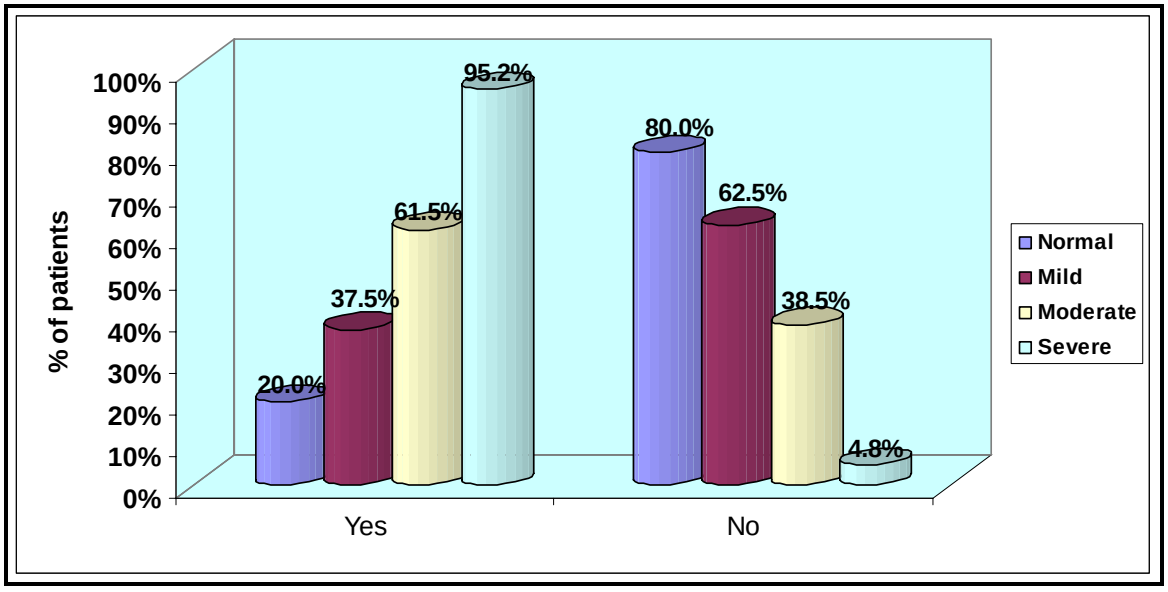


Fig 4: Blood pressure distribution (mm hg)

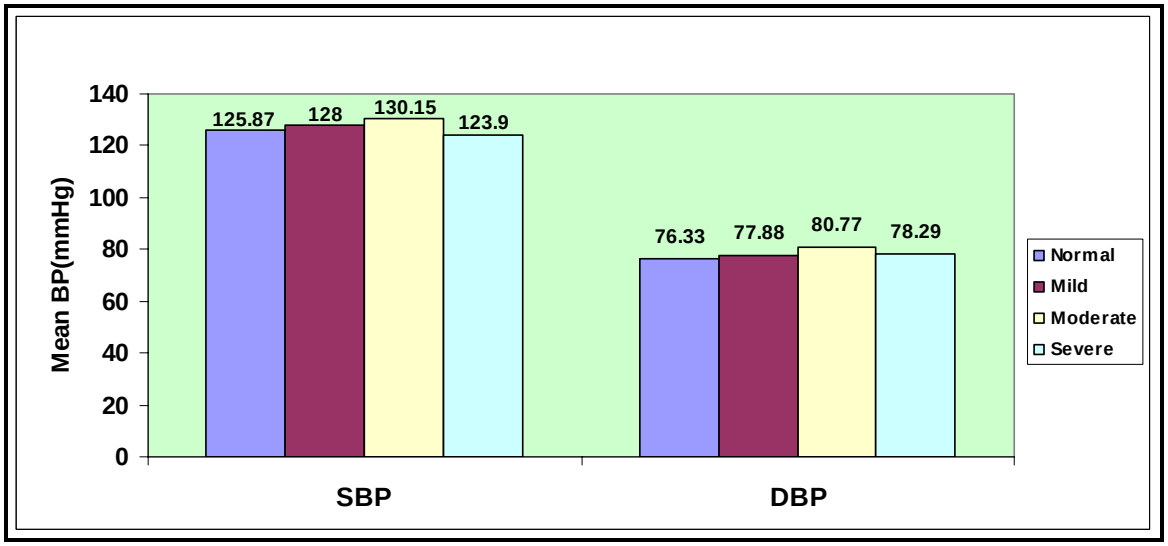


Fig 5: Leukocytes distribution (cu mm)

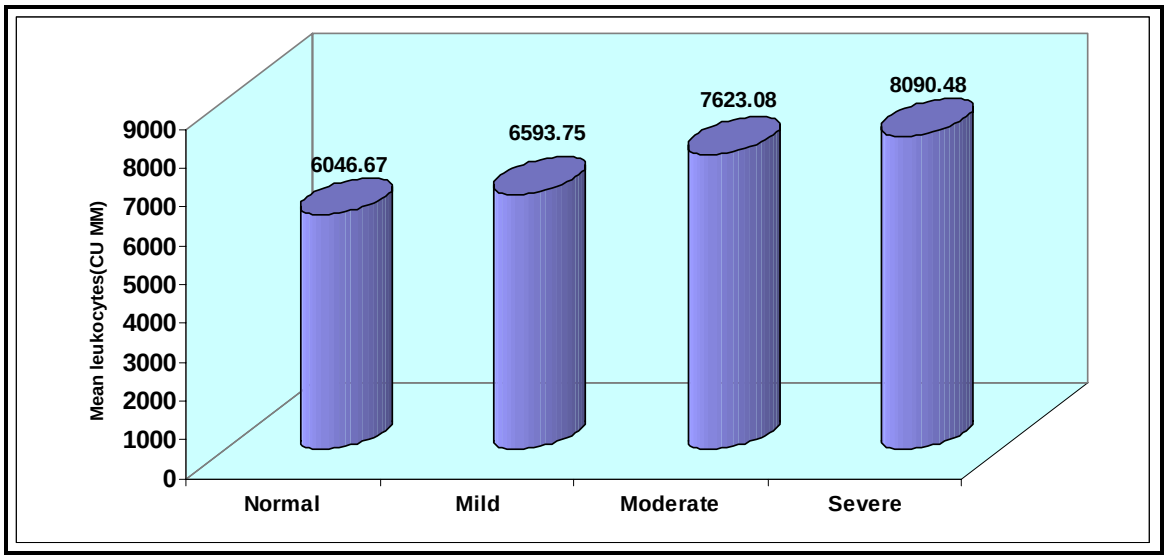


Fig 6: Neutrophils distribution (cu mm)

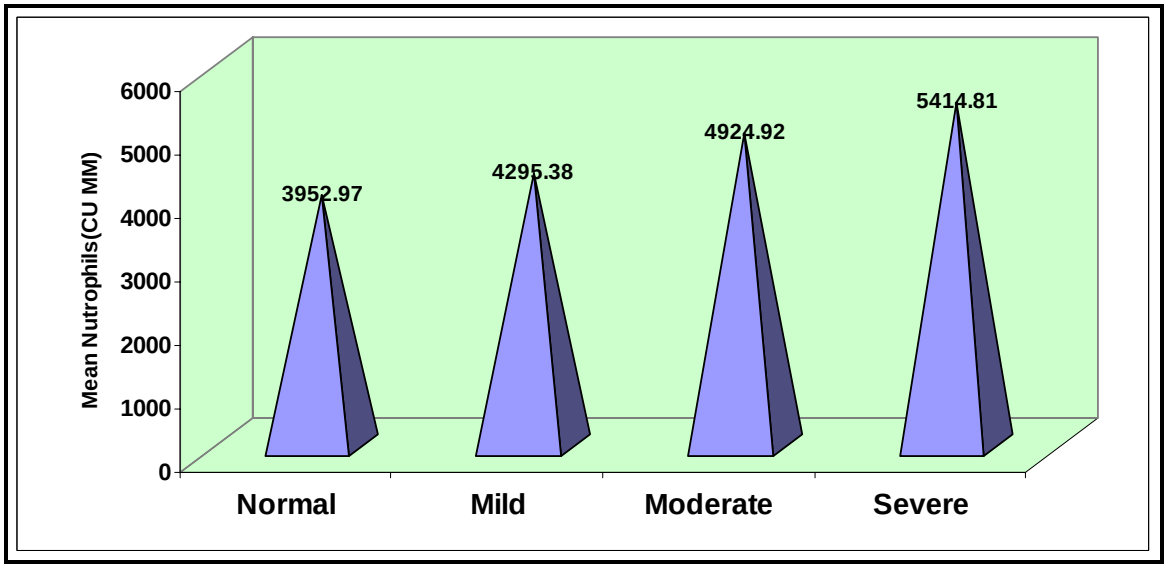


Fig 7: Lymphocytes distribution (cu mm)

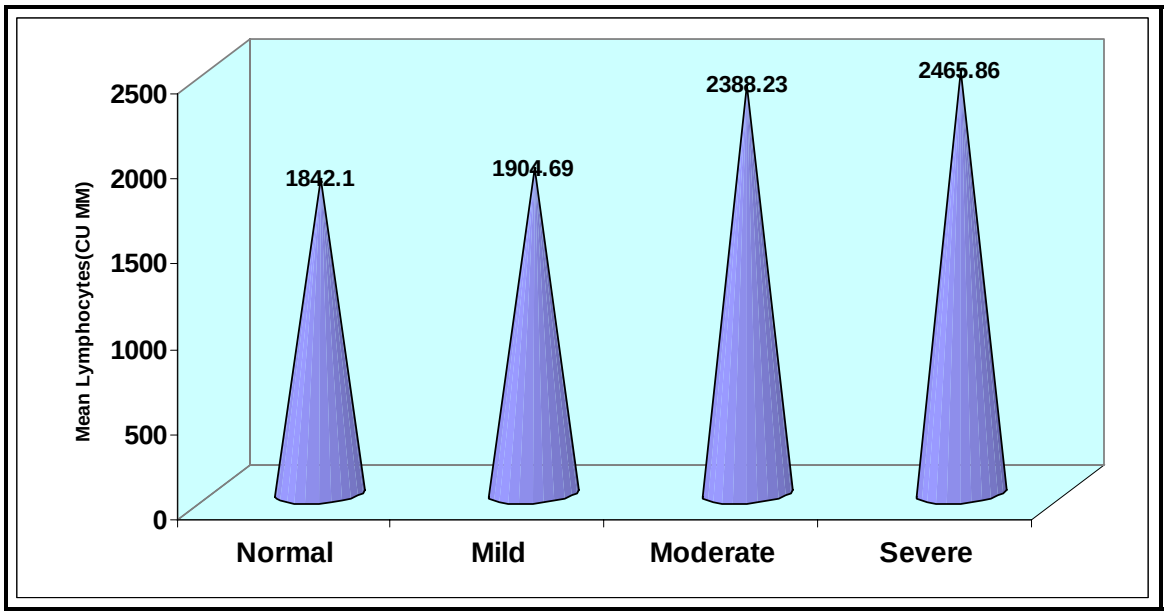


Fig 8: Platelets distribution (lakhs/cu mm)

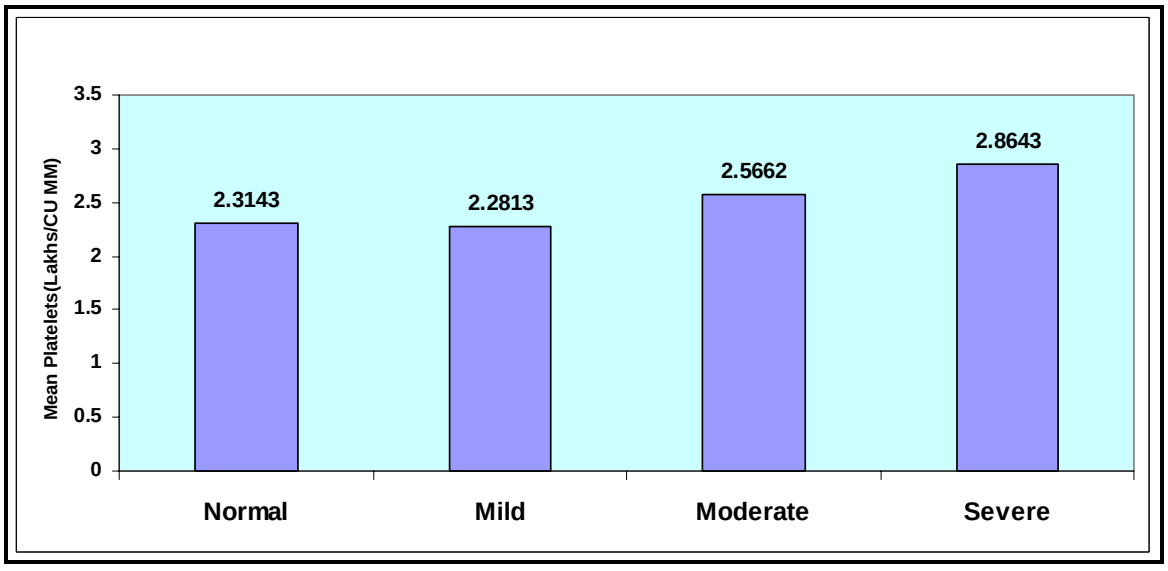


Fig 9: CRP distribution (mg/l)

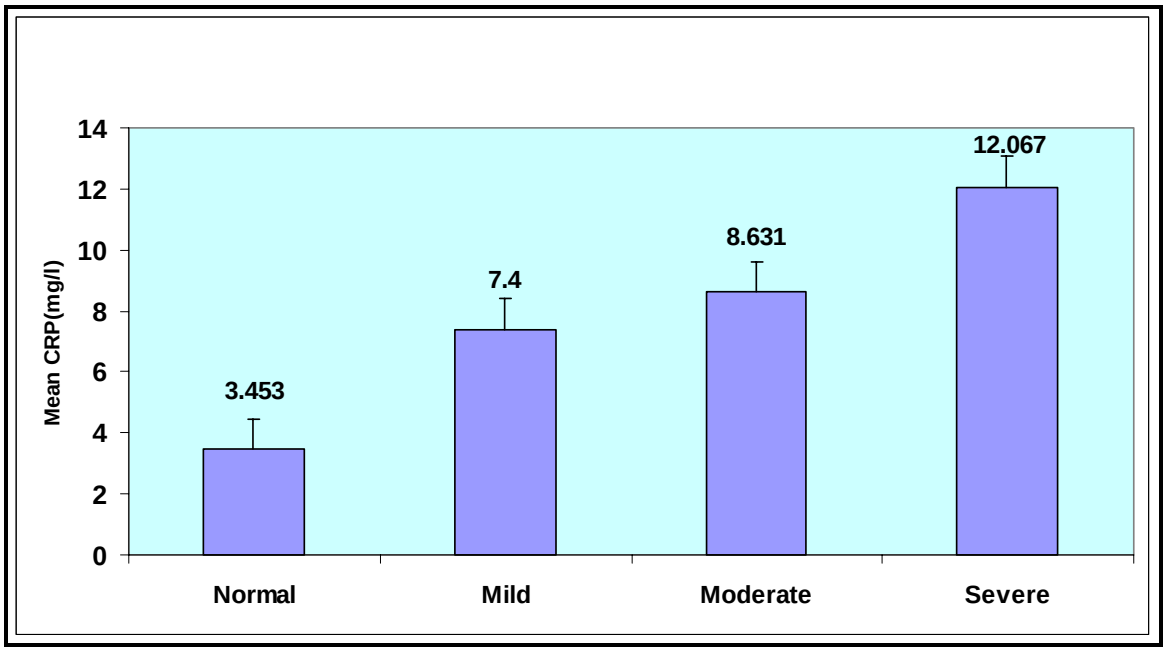
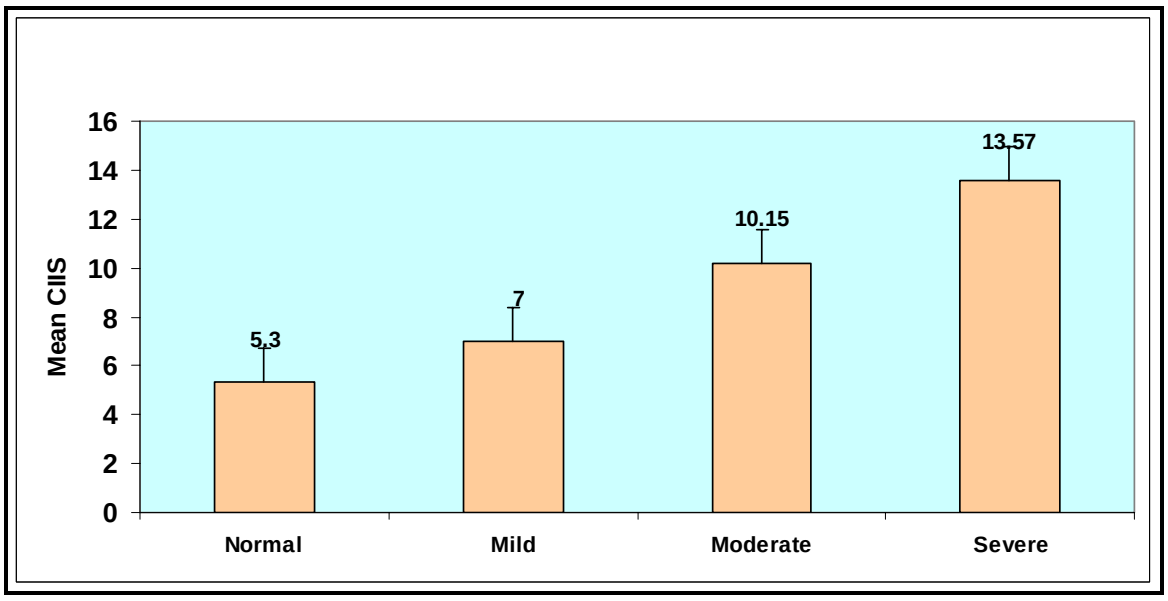


Fig 10: CIIS distribution



DISCUSSION

Many several relevant observations that may have important implications for future COPD management and research were made in this study. First, although previous studies have shown that severe COPD patients have elevated circulating levels of tumor necrosis factor, endothelin-1, interleukin-6, and CRP compared with healthy controls, these studies were limited by restriction of study subjects largely to those with severe COPD (FEV1 >50% of predicted)[58,64,77,78]. Our present study extends these findings by demonstrating the presence of systemic inflammation even in moderate (FEV1 50% to 80%) COPD. These data may explain why even relatively small reductions in FEV1 can increase the risk of cardiovascular morbidity and mortality 2- to 3-fold in the general community[3-7].

Second, although prior studies have shown systemic inflammation in severe COPD, [58,64,77,78] only few studies have established a link between systemic inflammation in COPD and cardiac injury. Our findings indicate that airflow obstruction is an important risk factor for cardiac injury. Even moderate obstruction is associated with elevated CIIS compared to healthy controls. In the presence of elevated CRP, this risk increases, which

suggests an important interplay of systemic inflammation with airflow obstruction in the development of ischemic heart disease.

Our study demonstrated that smoking was significantly associated with severity of airflow obstruction rather than the age. This shows that it is the duration of the smoking which is more important than age in determining the severity of COPD and its complications.

Our observations are similar to those of Sin et al[69], who showed the association of systemic inflammation and increased cardiovascular injury in COPD patients. They used the data from the third National health and nutrition examination survey (NHANES III).Their analysis was restricted to participants >50 years of age unlike our study where the lower limit of age was 45 years.

In our study females formed only 20% of those with airflow obstruction, whereas in the study done by Sin et al[69], percentage of females with airflow obstruction was almost equal to that of males (46.4 %). This could be explained by increased prevalence of smoking in western females. But male sex was a positive risk factor for severe airflow obstruction in their study which was similar to our study.

Our study also showed that smokers had an increased severity of obstruction, systemic inflammation and cardiovascular injury compared to nonsmokers which was similar to Sin et al[69] study. But even among non smokers low grade systemic inflammation was seen in those with chronic airflow limitation.

Study done by us showed that BMI is inversely correlated with severity of airflow obstruction similar to that of Sin et al[69].This is due to increasing levels of inflammatory cytokines as the severity of airflow obstruction increases.

Our study did not show significant differences in systolic blood pressure among the four groups. But Study done by Sin et al[69] showed that systolic blood pressure was elevated in those with severe airflow obstruction compared with controls. But no differences were noted between the other lung function groups. Significant differences were not observed in diastolic blood pressure in both the studies.

Our study showed that moderate and severe airflow obstruction was associated with increased levels of circulating leukocyte, platelets, and CRP.

This was similar to the study done by Sin et al[69] who showed that participants with severe and moderate airflow obstruction had increased levels of these inflammatory markers. Mild airflow obstruction was not associated with elevations in any of these measurements in both the studies.

Compared with the controls both studies demonstrated that moderate and severe airflow obstructions were associated with an increase in CIIS.

Our observations are also similar to those of Cirillo et al[79], who showed an inverse association between FEV1 and serum CRP. Unlike our study, however, they included all adult NHANES participants (increasing the risk of confounding by age). They also did not use a prediction equation for FEV1 to adjust for differences in age, sex, height, and race of study participants, which could have led to residual confounding by these variables. More importantly, the study by Cirillo et al[79] did not evaluate the role of airflow obstruction (or its severity) on systemic inflammation. Our study has extended the findings of Cirillo et al[79] and has shown that moderate to severe (but not mild) COPD is strongly associated with systemic low-grade inflammation and ECG evidence of ischemic heart disease.

Similar study was conducted by Miller et al and they also showed that reduced FEV1 seen in COPD was associated with increased cardiovascular risk (FRS, r=0.34, p<0.0001), and cardiac injury (CIIS, r=-0.34, p<0.0001).Cardiac injury scores were higher in patients with COPD than in healthy controls (8, 8±0.9vs.4.0±1.8units,p<0.04) and were greatest in patients with the most severe airflow obstruction (9.8±1.3units). CRP concentration increased with progressive airflow obstruction (2.0±0.4, 4.7±1.2, 5.2±0.7, 9.5±2.6 mg/L, FEV1 quartiles).

CRP was concentrated in the present study because it has been shown to upregulate the production of proinflammatory cytokines and tissue factors by monocytes, increase the uptake of LDL by macrophages, and directly induce expression of adhesion molecules by human endothelial cells. Additionally, CRP may deposit directly into the arterial wall during atherogenesis, interacting with other inflammatory mediators to create foam cells, which serve as building blocks of atherosclerotic plaques[80]. Leucocytosis and thrombocytosis also promote plaque formation but likely through different pathways[81]. Because neutrophilic inflammation may destabilize atherosclerotic plaques, leading to their rupture, our finding of increased circulating neutrophils among participants with severe airflow obstruction also may be relevant[82].

STUDY LIMITATIONS

1. The study is a hospital based study and may not be representative of general population.

2. As a cross-sectional study, the present analysis is limited in its ability to elucidate causal relationships between risk factors and outcome.

3. It is not known whether the reduction of proinflammatory markers would improve prognosis in COPD.

4. There may be alternate mechanistic pathways that may be responsible for cardiovascular complications in COPD.

5. Medication effects on circulating CRP and other inflammatory markers should also be considered.

CONCLUSIONS

1. The prevalence of COPD is high in males compared to females.

2. BMI is inversely correlated with the severity of the COPD.

3. COPD is associated with increased circulating levels of CRP and other inflammatory markers.

4. Intensity of the systemic inflammation increases with increase in the severity of airflow obstruction.

5. Airflow obstruction is associated with cardiac injury in patients with COPD.

SCOPE FOR FUTURE STUDIES

This study conducted in our Indian population has significant observation and potential implications. Our study concluded that COPD is associated with systemic inflammation and cardiovascular injury. Future studies should determine the absolute risk for developing coronary heart disease in patients with COPD. Future studies are also needed to determine if certain therapies that reduce the burden of systemic inflammation can lead to improved cardiovascular outcomes in COPD.

It is also necessary to determine what role (if any) the autonomic nervous system plays in the pathogenesis of adverse cardiovascular events in COPD.

Data from the present study confer a plausible mechanism to explain the strong relationship between COPD and cardiovascular diseases. More importantly, they extend the current concept of COPD as a systemic inflammatory disorder (and not just an inflammatory disorder of the pulmonary system) and provide potential new therapeutic targets to reduce cardiovascular complication rates in COPD.

PROFORMA

Name:

Age:

Sex:

Occupation:

OP No:

Address:

Married Y/N:

Smoker Y/N:

History if smoking in Pack Years:

Symptoms: h/o daily cough and sputum production

h/o breathlessness

Co morbidities: h/o Diabetes mellitus

h/o hypertension

h/o congestive heart failure

h/o tuberculosis

Drugs on use: bronchodilators

Inhaled or oral steroids.

Lipid lowering drugs

NSAID

Estrogen or progesterone products

Cardiac glycoside use

Family H/o DM/HT/ CAD/ CVA

EXAMINATION

Height weight BMI

Pulse peripheral pulses temperature

BP (upper limb)

CVS RS Abd CNS

INVESTIGATIONS

Spirometry: mild moderate severe obstruction

Serum inflammatory mediators: leukocytes lymphocytes

Neutrophils platelets

Hs-CRP

Blood glucose- Fasting postprandial

Lipid profile: TC TGL HDL LDL

Sputum for AFB:

ECG: sinus rhythm probable prior MI

CIIS LVH

Right axis deviation RVH

Simplified Cardiac Infarction Injury Score (CIIS)
Classifier for Practical Visual Coding of Electrocardiograms

Component	Lead	Feature	Threshold	score
1	aVL	Q duration in seconds (measured to near-est threshold)	Q absent 0.010 0.020 0.030 0.040 0.050	5 1 3 9 10 12
2	aVL	T amplitude in mm If T negative add 2 points for each mm	≤ 0.5 or ≥ 3 2	3
3	-aVR	R amplitude in mm = R (subtract 1 point for each mm)	-1	-R
4	-aVR	T amplitude (positive phase) in mm. Sub-tract 2 additional points for each mm exceeding 4	0 1 2 3 4	6 3 0 -2 -5
5	II, aVF	Largest Q:R ampli-tude ratio	≥ 1/20	12
6	III, -aVL	Largest Q duration in Seconds	≥ 0.040	5
7	III	T amplitude (negative phase) in mm	> 1	5
8	V1	T amplitude (positive phase) in mm	>2	5
9	V2	R amplitude in mm	< 3 or ≥ 14	5
10	V2	T amplitude (negative phase) in mm	≥ 1/4	5
11	V3	Q:R amplitude ratio	> 1/20	9
12	V5	S amplitude in mm	< 2	5

CIIS severity levels: level A, CIIS 20, probable injury;
level B, CIIS 15, possible injury; level C, CIIS 10, borderline abnormality.

MASTER CHART

COPD GROUP (CASES)

SL	SEX	AGE	SMO-KER	SMOK-ING	BP	BP	BMI		SPIROMETRY		LEUKO-CYTES	LYMPH-OCYTES	NEUTR-OPHILS	PLATELETS	HS-CRP	CIIS
		YRS		PACK YRS	MM HG	MM HG	KG/M2	MILD OBS	MOD OBS	SEVERE OBS	CU MM	CU MM	CU MM	LKS/CUMM	MG/L	
					SYSTOLIC	DIASTOLIC		FEV1>80%	FEV1 50-80%	FEV1<50%						
1	M	58	-	-	140	80	27.3			40	8900	2581	6141	2.95	10.8	11
2	M	50	+	12.5	124	80	18.65	84			7300	2190	4600	2.5	7.2	4
3	M	48	+	25	130	80	13.52			41	10800	2700	7776	3.1	10.6	14
4	M	65	+	120	120	80	18.75		58		7800	2106	5382	2.56	8.2	15
5	M	73	+	50	134	70	16.66			45	8400	2436	5796	2.6	11	10
6	M	52	+	10	130	70	16.65	90			7100	2556	4260	2.3	7	7
7	M	50	+	7.29	110	80	16			32	7900	2370	5372	2.8	24	13
8	M	51	+	12.5	110	80	16.02			30	8100	2349	5670	2.8	12	21
9	M	60	+	40	134	80	23.85			33	7600	2280	5168	2.95	10.2	17
10	M	55	+	3.75	100	70	20.23			32	8800	2816	5808	2.65	10	17
11	M	47	+	15	110	70	19.14			30	7900	2133	5451	3.1	16	22
12	M	58	+	30	140	70	22.2		65		7400	2220	5180	2.5	8.2	11
13	M	53	+	30	100	70	20			40	7800	2886	4680	3.15	10.4	12
14	M	45	+	12.5	110	70	16.4		70		8200	2624	4510	2.65	7.6	9
15	M	53	+	25	130	80	17.1			42	8000	2400	5520	2.9	10.6	14
16	M	49	+	25	134	84	21.48			37	7800	2184	5460	3	9.8	11
17	M	81	+	90	130	80	22.89			33	8600	3440	5160	2.2	10.4	16
18	M	65	-	-	124	80	20.34	83			7600	584	4560	1.45	6.4	14
19	M	48	-	-	134	80	21.4	86			5600	2016	3360	1.95	7.6	10
20	M	67	+	8	136	80	23.87		54		8000	2720	5120	2.6	7.6	11
21	M	68	+	40	140	90	27.73		70		7600	2584	4864	2.4	8	6
22	M	57	-	-	130	80	22.66	88			5600	1400	3920	2	7.3	3
23	M	55	+	25	130	90	18.96			32	7400	2220	4810	2.6	10.4	11
24	M	57	+	12.5	140	90	18.96		58		6600	2376	3960	2.65	8.2	12
25	M	57	+	13.33	124	80	14.45			34	7800	2340	5304	2.9	11	12
26	M	48	+	25	120	90	24.09			40	7600	2584	4940	2.8	12.2	11
27	M	65	-	-	130	70	16.45		74		8000	2800	4800	2.5	8.2	9
28	M	65	+	40	140	80	18.46			30	6500	1820	4485	3	11	14

29	M	47	+	27	106	70	18.36			33	8600	2752	5590	3	11.2	13
30	M	63	+	25	130	80	22.59			45	7600	2508	4560	2.75	10	10
31	M	60	+	16.66	130	80	22.83			32	8000	2400	5440	2.9	9.6	14
32	M	54	+	25	136	70	19.65	90			7000	2170	4690	2.3	4.2	6
33	M	59	+	20	124	80	21.25	85			6500	1820	4550	2.4	7	7
34	M	50	-	-	130	80	20.5		56		7200	2376	4680	2.5	8	10
35	M	64	+	40	130	84	18.6		54		6900	2070	4485	2.4	9	9
36	M	52	+	35	120	84	18.4		54		8000	2400	5200	2.6	10	10
37	M	50	+	20	120	70	21.7	83			5700	1596	3990	2.2	8.2	6
38	M	58	+	50	130	80	18.2			36	8000	2400	5120	2.9	18.2	12
39	M	70	+	15	124	80	19.8	81			6600	1914	4356	2.4	7.2	8
40	M	54	+	30	130	70	22.6			42	7800	2184	5460	3.1	14	10
41	F	53	-	-	118	70	17.7	85			5500	1650	3575	2.5	8.6	7
42	F	58	-	-	134	76	28	84			7000	1960	4760	2.4	7.9	5
43	F	60	-	-	136	80	22	83			6900	2070	4485	2.5	10	8
44	F	50	-	-	124	80	20		60		7500	2400	4950	2.8	9.8	10
45	F	58	-	-	130	80	18.66	90			7100	2343	4544	2.5	8.4	4
46	F	55	-	-	136	86	25.5		65		8000	2080	5600	2.5	9.2	9
47	F	56	-	-	126	80	16.65	92			5800	1740	3770	2.2	8	6
48	F	64	-	-	134	90	20	83			7000	2450	4410	2.4	5.6	8
49	F	53	-	-	124	80	19.68	82			7200	2016	4896	2.5	7.8	9
50	F	72	-	-	136	86	26.24		62		7900	2291	5293	2.7	10.2	11

CONTROLS

SL	SEX	AGE	SMOKER	SMOK-ING	BP	BP	BMI	SPIROMETRTY	LEUKOCYTES	LYMPHOCYTES	NEUTROPHILS	PLATELETS	HS - CRP	CIIS
		YRS		PACK YRS	MM/HG	MM/HG	KG/M2		CUMM	CUMM	CUMM	LKS/CUMM	MG/L	
					SYSTOLIC	DIASTOLIC								
1	M	52			124	70	26.4	N	5600	2128	3360	2.4	3	4
2	M	55			134	60	25.2	N	6000	2100	3720	2.1	2.6	4
3	F	50			114	74	22.6	N	5800	1740	3770	2.6	2	6
4	M	56			134	84	27	N	7000	2660	4060	2.2	6	5
5	M	58			120	70	26.4	N	6400	1792	4352	2.5	7.2	3
6	M	48			114	80	24.6	N	5100	1632	3264	2.1	4	5
7	F	53			130	80	22.3	N	6000	1680	4200	2.3	5.4	4
8	F	60			124	84	27.4	N	5700	1995	3591	2.15	2.4	6
9	F	58			136	90	24.5	N	6200	1860	4340	2.6	1.5	8
10	M	64	+	25	130	80	23.5	N	5900	1770	3835	2.18	3.4	4
11	M	50	+	20	124	76	26.4	N	5400	1620	3564	2.5	1.2	5
12	F	52			130	74	28.2	N	5600	1568	3808	2.4	6	7
13	F	56			124	80	24.6	N	7200	2304	4536	2.6	3	3
14	M	55	+	20	132	84	26.8	N	5800	1508	4060	1.9	2.4	4
15	F	50			114	60	23.4	N	6000	1800	3900	2.2	1	5
16	F	48			124	80	26.6	N	6100	1525	4270	2.4	2.8	6
17	F	54			130	70	25.5	N	5500	1650	3575	2.3	3.2	3
18	M	74			134	86	22.4	N	5800	1972	3480	2.6	8	4
19	F	62			124	60	24.2	N	5200	1352	3536	2.2	2.3	7
20	F	56			120	80	22.6	N	4800	1392	3168	2.1	1.6	5
21	M	54	+	21.6	134	80	26.3	N	8600	2580	5676	2.3	6.4	7
22	F	53			124	70	26.5	N	5900	1888	3599	2.5	2.3	4
23	F	50			114	80	25.4	N	6000	1800	3900	2.3	4.2	5
24	M	56	+	21.4	124	68	18.6	N	8000	2240	5360	2.1	3.6	8

25	F	66			130	80	24.8	N	6300	2079	3969	2.6	4.5	8
26	F	54			124	80	23.2	N	6100	1708	4209	2	2	5
27	M	63	+	19.6	130	80	26.8	N	6100	1952	3965	2.2	3	6
28	M	54			124	76	24.5	N	5500	1430	3850	2.4	2	4
29	M	50			130	80	26	N	5800	1798	3712	2.2	3.4	9
30	M	53			126	74	25.2	N	6000	1740	3960	2.5	3.2	5

ABBREVIATIONS

1. COPD: Chronic obstructive pulmonary disease.
2. GOLD: Global initiative for chronic obstructive lung disease.
3. FEV1: Forced expiratory volume in 1 second.
4. FVC : Forced vital capacity.
5. IL : Interleukins.
6. TNF : Tumor necrosis factor.
7. TGF : Tumor growth factor.
8. MMP : Matrix metalloproteinases.
9. TEAC : Trolox-equivalent antioxidant capacity.
10. CRP : C-Reactive protein.
11. BMI : Body Mass Index.
12. CIIS : Cardiovascular Infarction Injury Score.
13. Hs-CRP: High sensitive C-Reactive protein.

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